Avidin, a Potential Biopesticide and Synergist to *Bacillus thuringiensis* Toxins Against Field Crop Insects

YU CHENG ZHU, JOHN J. ADAMCZYK, JR., AND SANDY WEST

Southern Insect Management Research Unit, USDA–ARS–JWDSRC, P.O. Box 346, Stoneville, MS 38776

J. Econ. Entomol. 98(5): 1566–1571 (2005)

ABSTRACT A meridic diet was supplemented with avidin at various concentrations to determine its effects on growth and mortality of three lepidopteran insects: *Helicoverpa zea* (Boddie), *Spodoptera exigua* (Hübner), and *Anticarsia gemmatalis* (Hübner). All insects were placed on diet immediately after hatching and observed until death or pupation occurred. At a concentration of 10 ppm, avidin had little or no effect on growth and mortality compared with the control. However at a concentration of 100 ppm almost all tested insects were killed. *H. zea* was further tested by adding sublethal concentrations of Bt (Cry1Ac) in the diet containing avidin. The synergistic effect was significant, with mortality increasing to 44.4% over additive mortality (21.6%) of Bt and avidin.

KEY WORDS Bt, Biotin, synergism, Lepidoptera

TRANSGENIC CROPS HAVE HAD a profound impact on agriculture and the environment. *Bacillus thuringiensis* (Bt) cotton that produces toxins from the soil bacterium *Bacillus thuringiensis* Berliner is widely grown to control many important lepidopteran species. However, the potential evolution of Bt resistance in lepidopteran cotton pests (Gould et al. 1997, Gahan et al. 2001) could rapidly decrease the value of this biotechnology. To prolong the benefit of this biotechnology, alternative control measures should be developed to relieve selection pressure and slow down resistance development among many lepidopteran insects. Limited research has been conducted to increase Bt toxicity to *Helicoverpa armigera* (Hübner) and the diamondback moth, *Plutella xylostella* (L.) (Wang et al. 1999, Liu et al. 2000, Qiu et al. 2002). In these studies, up to a three-fold increase in toxicity was obtained by the addition of inorganic additives.

Avidin is a bioactive glycoprotein found naturally in the egg white of bird, reptile, and amphibian eggs. Chicken egg white contains no >500 ppm of avidin, which tends to be less stable and vulnerable to degradation by proteinases under acidic conditions in the human stomach (Kramer 2004). Transgenic corn seeds contain as much as 3000 ppm avidin (Kramer 2004). Avidin corn was not toxic to mice when administered as the sole component of their diet for 21 d (Kramer 2000). Avidin has a very strong affinity for the vitamin biotin, which is a coenzyme required for enzymes that catalyze carboxylation, decarboxylation, and transcarboxylation reactions in all forms of life (Kramer 2004). Avidin has a long history of use in a variety of biochemical and medical diagnostic procedures. Insecticidal activity of chicken avidin has been known since 1959 (Levinson and Bergmann 1959). Sequestration of biotin causes vitamin deficiency and in turn leads to stunted growth and mortality of many insect species. Transgenic avidin corn (Kramer et al. 2000), tobacco (Markwick et al. 2003), and apple (Markwick et al. 2003) plants showed strong resistance and insecticidal activities against many field crop and stored product insects. To explore the potential use of avidin for controlling a wide range of lepidopteran pests, we incorporated various concentrations of avidin into artificial diet to study its effect on three major noctuid insects. We also treated bollworm, *Helicoverpa zea* (Boddie), with avidin and Cry1Ac Bt toxin to investigate the synergistic effect between these two insecticidal proteins.

Materials and Methods

Effects of Avidin across Lepidopteran Species. Three lepidopteran species were selected for bioassays with avidin. These included bollworm; beet armyworm, *Spodoptera exigua* (Hübner); and the velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner). Eggs of all three lepidopteran species were supplied by the rearing laboratories of the USDA–ARS, Southern Insect Management Research Unit, Stoneville, MS. To determine the effect of avidin on larval mortality, larvae were reared on artificial diet. One liter of diet contains 120.0 g of dry-mix (tobacco budworm

Mention of a trademark, warranty, proprietary product or vendor does not constitute a recommendation or endorsement by the USDA and does not imply approval or recommendation of the product to the exclusion of others that may be suitable.

mix) (BioServe, Frenchtown, NJ), 20.0 g of ultrapure-low melting point agarose (Invitrogen, Carlsbad, CA), and 9.0 g of USDA vitamin premix (BioServe). To minimize mold, an acid mixture (2.5 ml) consisting of 42% propionic acid (A258, Fisher Scientific, Pittsburgh, PA), 4% phosphoric acid (04107, Sigma, St. Louis, MO), and 54% distilled water was added. Based on preliminary experiments, the artificial diet was supplemented with avidin (A9275, Sigma) at two concentrations [10 and 100 ppm (fwt)]. Diet was cooled in a water bath to 33°C before the appropriate avidin concentrations were added. All species were reared at 26.5°C and 40–60% RH in a growth chamber. Larval mortality was initially determined at 5 d after treatment (DAT) and observed at 7 and 10 DAT. Three replications of 10 larvae were examined for each treatment. For each replicate, larval mortality for avidin treatments was corrected from the appropriate natural mortality using Abbott's Formula (Abbott 1925). Means for each treatment were compared using analysis of variance (ANOVA) and separated using the Fisher's protected least significant difference procedure at the $\alpha = 0.05$ level (PROC MIXED, Littell et al. 1996, SAS Institute 1997).

Activity of Avidin and Synergy with Bt against Bollworm. Because the bollworm is the major lepidopteran pest on Bt cotton (Williams 2005), the activity of avidin and its relationship with Bt was examined for this species. Bioassays were conduced for the bollworm exactly as described above, except that additional concentrations were included (15, 20, 40, 60, and 80 ppm). In addition, surviving larvae from the 10, 15, and 20 ppm concentrations were weighed at 5, 7, 10, and 12 DAT. This facilitated the selection of avidin concentrations for synergy studies with Bt, that demonstrated low mortality but substantial effects on larval weight (see below), Larval mortality for each concentration was corrected as described above (Abbott 1925) and analyzed with the PROC PROBIT option of SAS (SAS Institute 1997).

The same artificial diet used in the above experiments was supplemented with Bt (Cry1Ac protoxin from MVP II) (Monsanto Corp., Louis, MO) at concentrations of 10 and 20 ppb. As with avidin, Bt concentrations were determined for the bollworm through preliminary experiments that demonstrated low mortality but substantial effects on larval weight (data not shown). Neonates were placed on diet supplemented with avidin only, Bt only, or avidin + Bt and monitored every 2 to 3 d for changes in larval mortality and weight until either pupation or death. Bollworms were reared at 26.5°C and 40–60% RH in a rearing chamber. Three replications of 10 larvae were studied for each experiment. Larval mortality for each concentration was corrected as described above (Abbott 1925). Means for each treatment were compared using ANOVA and separated using the Fisher's protected least significant difference procedure at the $\alpha = 0.05$ level (PROC MIXED, Littell et al. 1996, SAS Institute 1997).

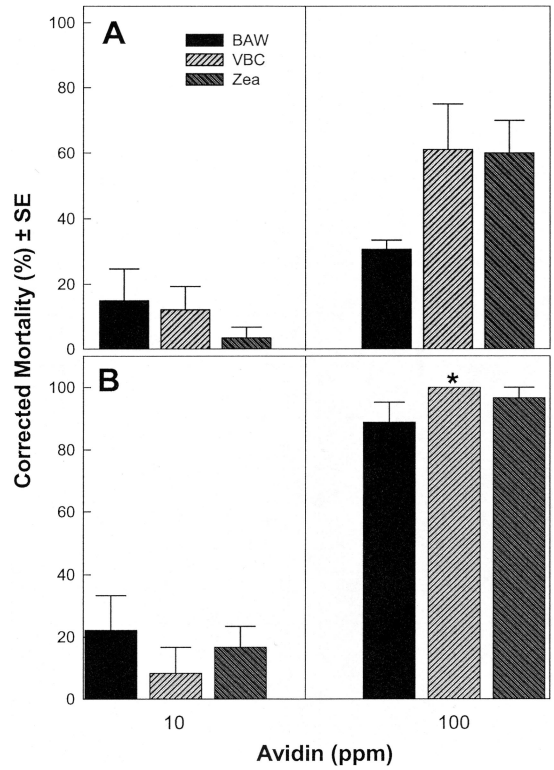

Fig. 1. Mortality of beet armyworm (BAW), velvetbean caterpillar (VBC), and bollworm (Zea) larvae when fed avidin incorporated into meridic diet at two concentrations. (A) 7 DAT. (B) 10 DAT. Regardless of species, mortality was significantly higher at 100 ppm than 10 ppm (7 DAT: $F = 44.14$; df = 1, 10; $P < 0.001$) (10 DAT: $F = 92.85$; df = 1, 10; $P < 0.001$). Asterisk (*) indicates 100% mortality observed at all replicates; therefore, it was excluded from the ANOVA.

Results

Effects of Avidin across Lepidopteran Species. The activity of avidin was similar across all three species (Fig. 1). For all treatments, control mortality never exceeded 20%. At 5 DAT, no significant differences ($P > 0.05$) in larval mortality were observed across the species and among the two treatments (10 and 100 ppm). In addition, at 7 and 10 DAT there were no significant differences ($P > 0.05$) in larval mortality among the three species and no significant interaction between species and treatments ($P > 0.05$). However, for all species, mortality was significantly higher ($P < 0.05$) when larvae were reared on diet containing 100 ppm compared with 10 ppm at 7 and 10 DAT. By 10 DAT, larval mortality for all species approached 100% on diet containing 100 ppm avidin.

Activity of Avidin and Synergy with Bt against Bollworm. A dose response with avidin was observed for the bollworm (Table 1). Mortality at 5 DAT was low, and a significant lack-of-fit did not allow for accurate calculation of fiducial limits. However, at 7 DAT and 10 DAT, observed mortality corresponded with predicted mortality that allowed LC_{50} values to

Table 1. Activity of avidin when incorporated into a meredic diet against *H. zea* larvae

Concn of avidin in diet (ppm)	7 DAT		10 DAT	
	Observed % mortality (SEM)[a]	Predicted % mortality	Observed % mortality (SEM)[a]	Predicted % mortality
0	0	.	0	.
10	3.3 (3.33)	7.80	16.7 (6.67)	8.91
15	13.3 (6.67)	13.28	13.3 (6.67)	24.93
20	26.7 (8.82)	18.45	33.3 (12.02)	42.01
40	33.3 (3.33)	35.27	96.7 (3.33)	82.71
60	46.7 (3.33)	47.06	96.7 (3.33)	94.66
80	56.7 (3.33)	55.65	96.7 (3.33)	98.16
100	60.0 (10.00)	62.16	96.7 (3.33)	99.30
Likelihood ratio χ^2	$P = 0.77$		$P = 0.032$[b]	
LC_{50} (95% FL)	66.20 (51.12–96.13)		22.60 (17.97–28.05)	
Slope (ln) (SEM)	0.75 (0.127)		1.65 (0.248)	

[a] Average of three replicates, 10 larvae per replicate.
[b] Large χ^2 ($P < 0.05$). A t-value of 2.09 used to compute 95% fiducial limits.

be calculated with relative certainty (7 DAT: LC_{50} = 66.2 ppm, 10 DAT: LC_{50} = 22.6 ppm). By 10 DAT, observed mortality approached 100% at concentrations >40 ppm.

At concentrations of avidin that produced low levels of mortality, significantly lower larval weights ($P < 0.05$) were observed compared with the untreated control (Fig. 2). Because a significant interaction ($P < 0.05$) was observed between time of evaluation and the four treatments ($F = 4.61$; df = 9, 30; $P < 0.001$), separate ANOVAs were conducted for each time of evaluation. At 5 DAT, significant differences among larval weights were observed ($F = 52.90$; df = 3, 8; $P < 0.001$). Larvae that were reared on diet containing 10 ppm weighed significantly more ($P < 0.05$) compared with larvae reared on higher concentrations of avidin or the untreated control. At 7 DAT, significant differences among larval weights were observed ($F = 29.61$; df = 3, 6; $P = 0.005$). Larvae that were reared on diet containing 10 ppm weighed significantly more ($P < 0.05$) compared with larvae reared on higher concentrations of avidin or the untreated control. However, larvae that were reared on diet containing the highest dose of avidin (20 ppm) weighed significantly less ($P < 0.05$) compared with larvae reared on all other doses of avidin or the untreated control. At 10 DAT, significant differences among larval weights also were observed ($F = 28.34$; df = 3, 8; $P < 0.001$). Larvae that were reared on diet containing 15 and 20 ppm weighed significantly less ($P < 0.05$) compared with larvae reared on diet containing 10 ppm or the untreated control. Furthermore, at 10 DAT there were no significant differences among larval weights ($P > 0.05$) for diet containing 10 ppm of avidin and the untreated control. At 12 DAT, significant differences among larval weights also were observed ($F = 5.87$; df = 3, 8; $P = 0.023$). However, only diet containing 15 and 20 ppm of avidin significantly reduced larval weights ($P < 0.05$) compared with the untreated control.

Avidin is synergized by the addition of Bt against the bollworm (Figs. 3 and 4). As observed for the abovementioned experiment involving weight response of

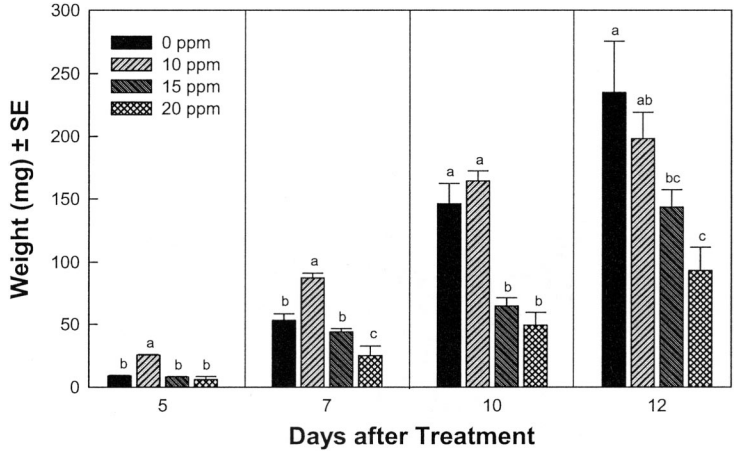

Fig. 2. Larval weights for bollworms fed avidin at sublethal doses ($<LC_{50}$) incorporated into meridic diet. Within a time period, bars with a common letter are not significantly different ($\alpha = 0.05$) from one another according to Fisher's protected least significant difference.

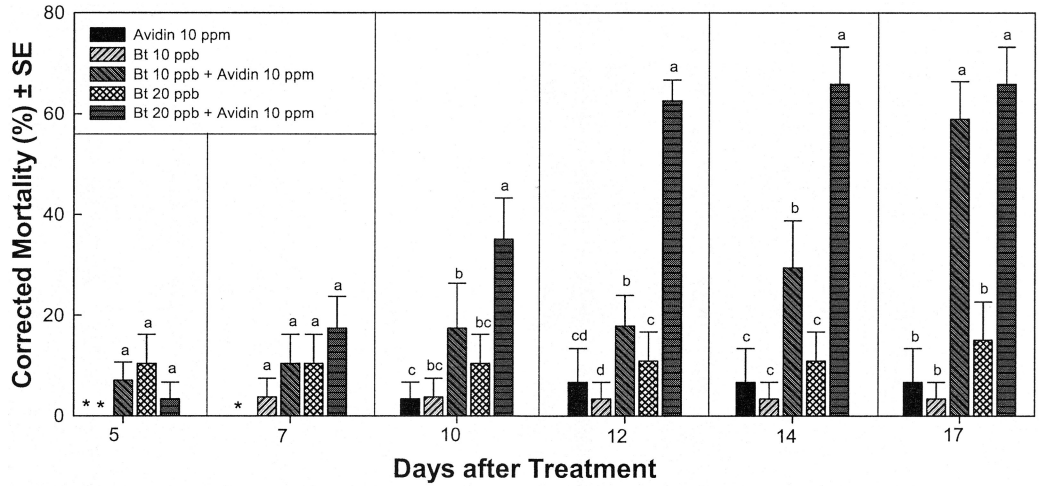

Fig. 3. Mortality of bollworm larvae when fed avidin, Bt, or both incorporated into a meridic diet. Within a time period, bars with a common letter are not significantly different ($\alpha = 0.05$) from one another according to Fisher's protected least significant difference. Asterisk (*) indicates 0% mortality observed at all replicates; therefore, it was excluded from the ANOVA.

avidin and the bollworm, a significant interaction ($P < 0.05$) was observed between time of evaluation and the treatments (larval mortality: $F = 15.44$; df = 17, 52; $P < 0.001$, and larval weights: $F = 13.64$; df = 15, 46; $P < 0.001$); therefore, separate ANOVAs were conducted for each time of evaluation. At 5 and 7 DAT, no significant differences ($P > 0.05$) in larval mortality were observed among the treatments (Fig. 3). However, at 10–17 DAT, significant differences in larval mortality ($P < 0.05$) among the treatments were observed (10 DAT: $F = 9.52$; df = 4, 8; $P = 0.004$, 12 DAT: $F = 127.63$; df = 4, 8; $P < 0.001$, 14 DAT: $F = 54.82$; df = 4, 8; $P < 0.001$, and 17 DAT: $F = 62.78$; df = 4, 8; $P < 0.001$). At 12, 14, and 17 DAT, larval mortality was significantly greater ($P < 0.05$) for avidin at 10 ppm containing Bt at both 10 or 20 ppb compared with all other treatments. In addition, larval weights were significantly different among the treatments for all time periods (5 DAT: $F = 16.41$; df = 5, 12; $P < 0.001$, 7 DAT: $F = 113.15$; df = 5, 12; $P < 0.001$, 10 DAT: $F = 55.77$; df = 5, 12; $P < 0.001$, and 12 DAT: $F = 25.01$; df = 5, 12; $P < 0.001$) (Fig. 4). Although not always significant, treatments containing both avidin and Bt had numerically lower weights compared with treatments containing avidin or Bt alone, or the untreated control.

Discussion

In this study, growth of all three lepidopteran insects was greatly retarded by diet that contained avi-

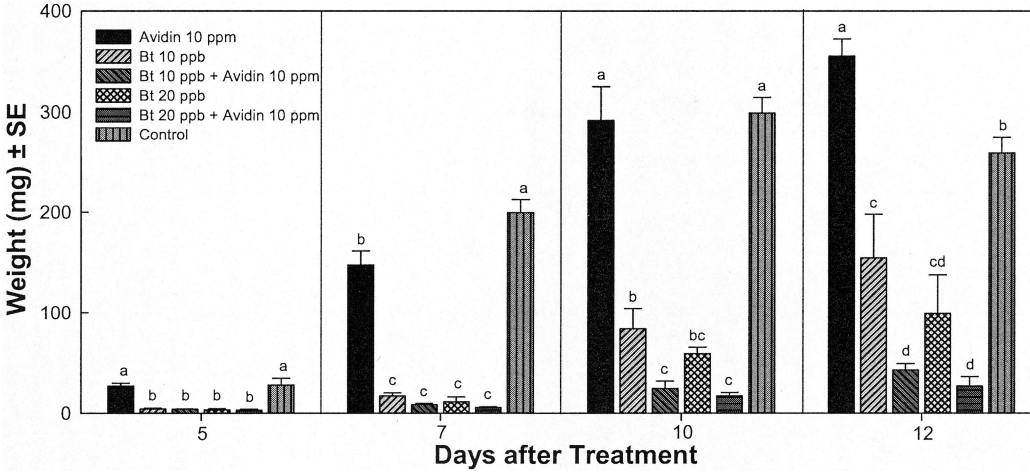

Fig. 4. Larval weights for bollworms fed avidin, Bt, or both incorporated into a meridic diet. Within a time period, bars with a common letter are not significantly different ($\alpha = 0.05$) from one another according to Fisher's protected least significant difference.

din. In addition, we also observed significant activity of avidin against the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) as well as the tobacco budworm, *Heliothis virescens* (L.) (data not shown). Avidin in diet at a concentration as low as 40 ppm could kill up to 100% of the larvae. We also found that avidin synergized Cry1Ac Bt toxicity against the bollworm, and our results are consistent with those of Burgess et al. (2002). Avidin is a bioactive protein, and the gene coding for its synthesis could be inserted into cotton (or other field crops) genomes alone or stacked with Bt genes. By targeting different sites, avidin and Bt together could be more effective in suppressing lepidopteran insects and also could help delay development of resistance to Bt.

Currently, in the mid-southern United States, transgenic Bt cotton makes up >90% of the cotton grown (Williams 2005). It is widely used because it effectively controls the tobacco budworm. In current transgenic (Bt) cotton systems, resistance development in a lepidopteran insect is a potential threat to the Bt biotechnology because of high selection pressure against target insects from one or dual highly specific toxins due to widespread adoption of Bt cotton. Bt resistance is attainable and a number of insect species have developed resistance through laboratory selection (Tabashnik 1994), including the tobacco budworm with >10,000-fold resistance (Gould et al. 1995) and the pink bollworm *Pectinophora gossypiella* (Saunders), with >3,100-fold resistance (Tabashnik et al. 2002). Resistance to transgenic Bt cotton also has been observed in *H. armigera* (Meng et al. 2004). Unlike classes of Bt toxins, avidin targets different binding sites. In the current study, the synergistic effect of avidin with the Cry1Ac Bt toxin against the bollworm (also seen with Cry1Ba against *H. armigera;* Burgess et al. 2002) suggests strong potential for stacking these genes into one transgenic plant. Incorporation of an avidin gene into the cotton genome alone or stacked with Bt toxin genes could be advantageous for Bt resistance management. Furthermore, bollworms could perhaps be completely controlled by avidin alone if cotton could express the toxin at levels comparable to corn [3,000 ppm as shown by Kramer (2004).

In the mid-southern United States, it is also very important to seek alternative insecticidal proteins to be inserted into the cotton genome for controlling not only lepidopteran pests but also sucking insects. The widespread use of transgenic Bt cotton along with boll weevil, *Anthonomus grandis grandis* Boheman, eradication has reduced the number of insecticide applications made to cotton each year. This has allowed formerly secondary insects, such as the tarnished plant bug and other sucking insects, to become more serious problems in cotton (Snodgrass 1996, Snodgrass and Scott 2000). Therefore, transgenic cotton with genes that target a wider range of pests is urgently needed. In addition to its efficacy against lepidopteran insects (Markwick et al. 2001, 2003; Burgess et al. 2002), avidin is a highly insecticidal against many coleopteran pests (Morgan et al. 1993, Allsopp and McGhie 1996, Kramer et al. 2000). If avidin also is shown to be effective against sucking insects, its introduction into transgenic cotton, along with its synergistic action with Bt toxins, could be a major advance in cotton insect control and Bt resistance management. In the future, we hope to develop artificial diet and an avidin delivery technique for bioassay with sucking insects.

Acknowledgments

We thank Gordon L. Snodgrass and Jeff Gore (USDA–ARS, Stoneville, MS) and Andrew Y. Li (USDA–ARS, Kerrville, TX) for reviewing and improving an earlier draft of this manuscript. In addition, we thank statistician, Debbie Boykin for assistance. We also appreciate Shawana Cox for assisting with certain experiments.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Allsopp, P. G., and T. K. McGhie. 1996. Snowdrop and wheatgerm lectins and avidin as antimetabolites for the control of sugarcane whitegrubs. Entomol. Exp. Appl. 80: 409–414.

Burgess, E.P.J., L. A. Malone, J. T. Christeller, M. T. Lester, C. Murray, B. A. Philip, M. M. Phung, and E. L. Tregidga1. 2002. Avidin expressed in transgenic tobacco leaves confers resistance to two noctuid pests, *Helicoverpa armigera* and *Spodoptera litura.* Transgenic Res. 11: 185–198.

Gahan, L. J., F. Gould, and D. G. Heckel. 2001. Identification of a gene associated with Bt resistance in *Heliothis virescens.* Science (Wash. DC) 293: 857–860.

Gould, F., A. Anderson, A. Reynolds, L. Bumgarner, and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88: 1545–1559.

Gould, F., A. Anderson, A. Jones, D. Sumerford, D. G. Heckel, J. Lopez, S. Micinski, R. Leonard, and M. Laster. 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens.* Proc. Natl. Acad. Sci. U.S.A. 94: 3519–3523.

Kramer, K. J. 2000. Putting a chicken gene into corn results in an insect-resistant transgenic grain, pp. FF1-FF4. *In* J. P. Cherry and A. E. Pavlath [eds.], Proceedings of the US-Japan Cooperative Program in Natural Resources, 19–25 Nov. 2000, Honolulu, HI.

Kramer, K. J. 2004. Avidin: an egg-citing insecticidal protein in transgenic corn, pp. 119–130. *In* G. H. Liang and D. Z. Skinner [eds.], Genetically modified crops: their development, uses, and risks. Haworth Press, Inc., Binghamton, NY.

Kramer, K. J., T. D. Morgan, J. E. Throne, F. E. Dowell, M. Bailey, and J. A. Howard. 2000. Transgenic avidin maize is resistant to storage insect pests. Nat. Biotech. 18: 670–674.

Levinson, J. N., and E. D. Bergmann. 1959. Vitamin deficiencies in the housefly produced by antivitamins. J. Insect Physiol. 3: 293–305.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS institute, Cary, NC.

Liu, M., J. Dai, and Z. Yu. 2000. Screening on chemical synergistic factors to Bt (*Bacillus thuringiensis*) oil formulation. J. Huazhong Agri. Univ. 19: 134–137.

Markwick, N. P., J. T. Christeller, L. C. Docherty, and C. M. Lilley. 2001. Insecticidal activity of avidin and streptavidin against four species of pest Lepidoptera. Entomol. Exp. Appl. 98: 59–66.

Markwick, N. P., L. C. Docherty, M. M. Phung, M. T. Lester, C. Murray, J.-L. Yao, D. S. Mitra, D. Cohen, L. L. Beuning, S. Kutty-Amma, et al. 2003. Transgenic tobacco and apple plants expressing biotin-binding proteins are resistant to two cosmopolitan insect pests, potato tuber moth and lightbrown apple moth, respectively. Transgenic Res. 12: 671–681.

Meng, F., J. Shen, W. Zhou, and H. Cen. 2004. Long-term selection for resistance to transgenic cotton expressing *Bacillus thuringiensis* toxin in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Pest Manage. Sci 60: 167–172.

Morgan, T. D., B. Oppert, T. H. Czapla, and K. J. Kramer. 1993. Avidin and streptavidin as insecticidal and growth inhibiting dietary proteins. Entomol. Exp. Appl. 69: 97–108.

Qiu, S., Z. Huang, B. Huang, and X. Guan. 2002. Effect of additives on *Bacillus thuringiensis* for controlling *Plutella xylostella.* Chin. J. Biol. Ctrl. 18: 62–66.

SAS Institute. 1997. User's manual, version 6.12. SAS Institute, Cary, NC.

Snodgrass, G. L. 1996. Insecticide resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. J. Econ. Entomol. 89: 783–790.

Snodgrass, G. L., and W. P. Scott. 2000. Seasonal changes in pyrethroid resistance in tarnished plant bug (Heteroptera: Miridae) populations during a three year period in the Delta area of Arkansas, Louisiana, and Mississippi. J. Econ. Entomol. 93: 441–446.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis.* Annu. Rev. Entomol. 39: 47–79.

Tabashnik, B. E., Y. Liu, T. J. Dennehy, M. A. Sims, M. S. Sisterson, R. W. Biggs, and Y. Carrière. 2002. Inheritance of resistance to Bt toxin Cry1Ac in a field-derived strain of pink bollworm (Lepidoptera: Gelechiidae). J. Econ. Entomol. 95: 1018–1026.

Wang, M., X. Yin, X. Guo, Y. Wu, and X. Jia. 1999. Study on synergism of addition of inorganic salts to *Bacillus thuringiensis* preparations preventing and curing *Helicoverpa armigera.* Acta Agric. Univ. Henanensis 33: 186–189.

Williams, M. R. 2005. Cotton insect loss estimates –2004. *In* P. Dugger and D. A. Richter [eds.], Proceedings of the 2004 Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Received 31 January 2005; accepted 22 June 2005.